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(54) **BIOCHEMICAL PURIFICATION OF SIMVASTATIN**

BIOCHEMISCHE REINIGUNG VON SIMVASTATIN

PURIFICATION BIOCHIMIQUE DE SIMVASTATINE

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- **CHEMICAL ABSTRACTS, vol. 113, no. 17, 22 October 1990, Columbus, Ohio, US; abstract no. 144802n, VICKERS, S. ET AL. 'In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase.' page 11 ;**
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Description

BRIEF SUMMARY OF THE INVENTION

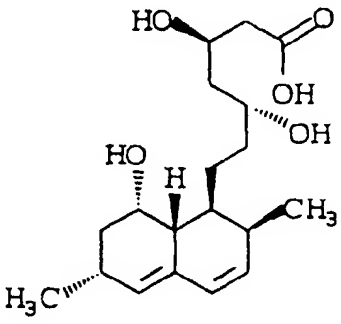
The present invention relates to biosynthetic conversion of lovastatin to 7-[1',2',6',7',8',8a'(R)-hexahydro-2'(S),6'(R)-dimethyl-8'(S)-hydroxy-1'(S)-naphthyl]-3(R),5(R)-dihydroxyheptanoic acid, "triol acid" by microbiological hydrolysis in the synthesis of simvastatin from lovastatin to facilitate the separation and isolation of simvastatin from unreacted lovastatin starting material. The process employs a bacterium or fungus capable of hydrolyzing the 2-methylbutyryloxy side chain of lovastatin, or a mutant of such a microbe or a hydrolase derived therefrom.

The triol acid and its lactone form are known in the art and are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol biosynthesis.

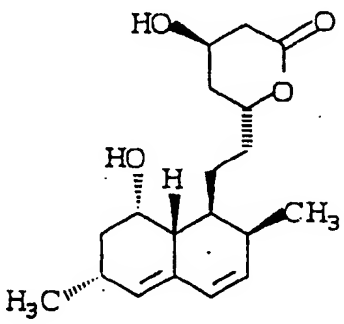
The selective conversion of lovastatin salt to the triol salt is useful for the separation of simvastatin from unreacted lovastatin in the production of simvastatin from lovastatin. Lovastatin acid has a 2-methylbutyryloxy side chain in the 8'-position and is difficult to separate from the newly formed simvastatin acid which has a 2,2-dimethyl-butyryloxy side chain at the 8'-position. Applicants have now found that selective cleavage of the 2-methylbutyryloxy side chain from lovastatin acid salt using the process of this invention employing a hydrolase enzyme from a microbe including fungi such as Clonostachys compactiuscula (ATCC 38009 and ATCC 74178), Monascus ruber, Mortierella isabellina, Emericella unguis, Diheterospora chlamydosporia, Humicola fuscoatra, Dichotomomyces cejpii, Neocosmospora africana, Xylogone sphaerospora, Torulomyces ragena, Thielavia fimeti, Aspergillus unguis, Mucor circinelloides, Fusarium solani, Penicillium chrysogenum, Aspergillus clavatus, Scopulariopsis communis, Gilmaniella humicola, Mucor bainieri, Tricharus spiralis, and Chaetomium cochliodes, or bacteria, especially an actinomycete such as Streptomyces albogriscolus, Streptomyces paucisporogenes, Streptomyces hygroscopius, Streptomyces viridochromogenes, Planomonospora parontospora, and Kibdelosporangium aridum to yield the triol salt results in a more easily separable mixture and greater purity of the simvastatin product.

BACKGROUND OF THE INVENTION

The present invention is in the field of inhibitors of HMG-CoA reductase which are useful as antihypercholesterolemic agents. It is now well established that hypercholesterolemia is a significant risk factor in the development of cardiovascular disease, particularly atherosclerosis. Compounds which are able to inhibit the HMG-CoA reductase enzyme interfere with and limit the biosynthesis of cholesterol, and in that way function as antihypercholesterolemic agents.



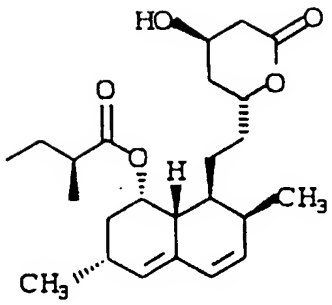
Triol Acid



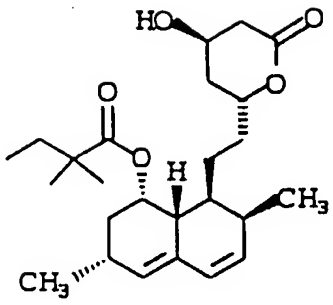
Diol Lactone

As already described above, the triol acid and its lactone form are old compounds. The triol acid in its lactone form, for example, is described in Endo, published Japanese Pat. Appln. 86-13798 (1986), where its production by fermentation of Monascus ruber and a demonstration of its ability to reduce blood cholesterol levels is also set out.

Lovastatin and simvastatin are also compounds known in the art as HMG-CoA reductase inhibitors. The two compounds differ in that lovastatin has a 2-methylbutyryloxy side chain at the 8'-position and simvastatin has a 2,2-dimethylbutyryloxy side chain.



Lovastatin



Simvastatin

Although simvastatin has been synthesized from lovastatin, it has been difficult to separate and purify simvastatin from a mixture of simvastatin and lovastatin. The similarity in structure between the two compounds (the two compounds differ by only one methyl group) makes high pressure liquid chromatography (HPLC) separation difficult because the compounds have such similar retention times. One methodology used to isolate simvastatin from a mixture of simvastatin and lovastatin is to convert the unreacted lovastatin to the triol acid or the diol lactone using base hydrolysis with, for example, sodium hydroxide (NaOH) or lithium hydroxide (LiOH). However, this base hydrolysis hydrolyzes only a percentage of the lovastatin, leaving unreacted lovastatin as a contaminant of the final simvastatin product. An additional problem with the base hydrolysis is partial hydrolysis of the simvastatin, thus reducing the yield of the desired simvastatin product. The present invention provides for a process of isolating simvastatin from mixtures of simvastatin and lovastatin in greater purity and without concomitant yield losses.

Komagata et al., J. Antibiotics, 39, 1574-77 (1986), describes enzymatic hydrolytic conversion of compactin (ML-236B) to the 8-hydroxy analog (ML-236A) in which the same side chain is removed as in the present invention. Of 1600 fungal strains investigated, 59 were found to be effective in catalyzing the hydrolytic reaction, and Emericella unguis showed the most potent activity.

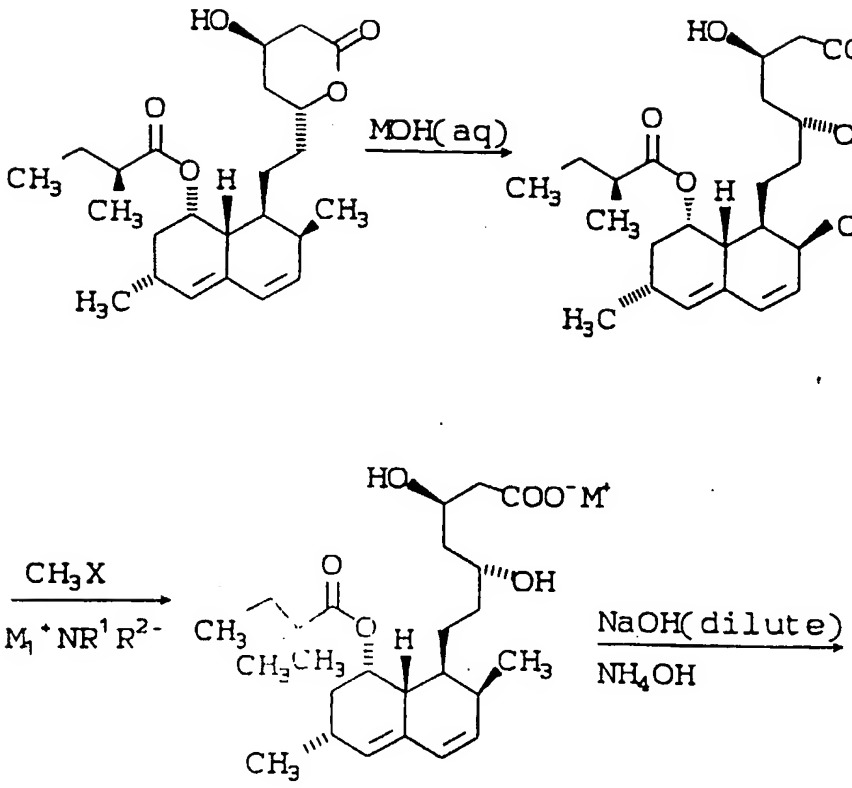
Endo, published Japanese Pat. Appln. 85-176595 (1985) describes the same conversion as Komagata et al. above, but additionally includes conversion of "monacolin K" (which is lovastatin) to "monacolin J", (which is the triol acid in the present invention). Especially useful are said to be the molds Mortierella isabellina, Emericella unguis, Diheterospora chlamydosporia, Humicola fuscoatra, Dichotomomyces cejpii, Neocosmospora africana, Xylogone sphaerospora, Torulomyces ragena, and Thielavia fimeti.

European Patent Publication EPO 486 153 teaches that Clonostachys compactiuscula ATCC 38009 is capable of converting lovastatin acid to triol acid. This same strain has also been redeposited with the American Type Culture Collection as ATCC 74178.

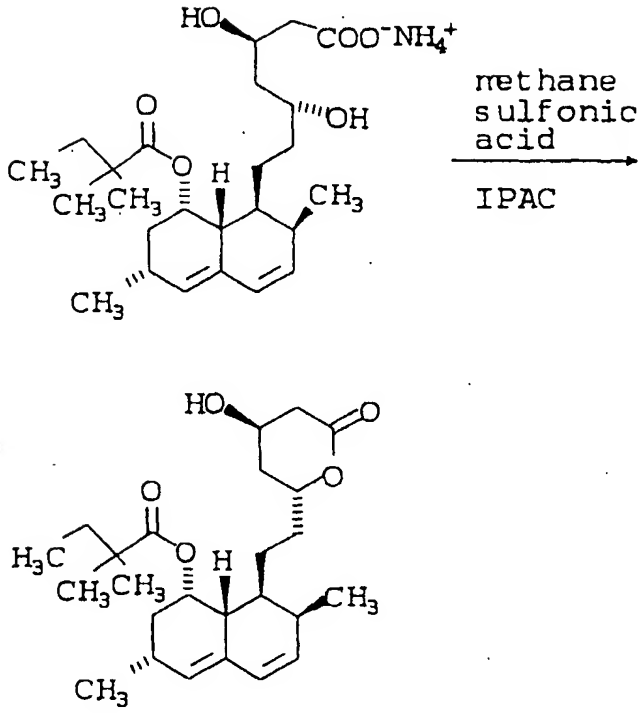
Lovastatin can be converted to a more active HMG-CoA reductase inhibitor by C-methylation of the natural 2(S)-methylbutyryloxy side chain to obtain simvastatin. C-methylation may be accomplished by any known process amenable to the functionalities of the molecule.

One process for direct C-methylation of the 2(S)-methylbutyryloxy side chain is described in U.S. Patent No. 4,582,915. This process is detailed in Scheme I and in the description which follows.

SCHEME I


HO
O
O
O
CH3
CH3
O
H
CH3
H3C
MOH(aq)
HO
COO- M+
OH
CH3
CH3
O
H
CH3
H3C
CH3X
M1+ NR1 R2-
HO
COO- M+
OH
O
CH3
CH3 CH3
O
H
CH3
CH3
NaOH(dilute)
NH4OH

SCHEME I (con't)



wherein:

M	is an alkali metal salt, preferably potassium;
X	is halo, such as chloro, bromo or iodo, preferably bromo or iodo;
M_1^+	is a cation derived from lithium, sodium or potassium, preferably lithium; and
R^1 and R^2	are

1) independently C_{1-3}alkyl, or
2) R^1 and R^2 joined together form a 5- or 6-membered heterocycle such as pyrrolidine or piperidine with the nitrogen to which they are attached, preferably pyrrolidine.

In the process of forming simvastatin by the direct methylation of lovastatin, the lovastatin lactone compound is first converted to an alkali metal salt, preferably a potassium salt of the dihydroxycarboxylate. Although any conceivable method for preparing a dry salt would suffice, it is convenient to add a substantially stoichiometric amount of aqueous potassium hydroxide to a solution of the lactone starting material in a hydrocarbon solvent such as benzene, toluene or cyclohexane containing a small amount of a C_{1-3} alkanol, preferably isopropanol, ethanol or methanol, or alternatively in tetrahydrofuran (THF) with or without added alkanol, stirring for a few minutes to about an hour and finally concentrating to dryness in vacuo. The residue is subjected to rigorous drying such as by azeotropic distillation with cyclohexane, toluene or dry tetrahydrofuran, preferably extremely dry (less than 0.08 mg H_2O/mL) tetrahydrofuran.

The dry alkali metal salt is dissolved in an ethereal solvent such as tetrahydrofuran, diethyl ether, 1,2-dimethoxyethane, cooled to about -80°C to -25°C and treated with an excess of a strong base such as an alkali metal amide, wherein the alkali metal is lithium, sodium or potassium, preferably lithium, and the amide is diethylamide, pyrrolidide, dimethylamide or diisopropyl amide in an ethereal solvent in a dry, inert environment. After about 2 to 8 hours, preferably

about two hours at -80° to -25°C, preferably -35° to -30°C, a methyl halide, such as methyl bromide, methyl chloride or methyl iodide, preferably methyl bromide or methyl iodide, is added to the mixture while maintaining the low temperature. Treatment with the strong base and methyl halide as described can be repeated if appreciable amounts of starting material remain. After 0.5 to about 3 hours following final addition of methyl halide, the reaction mixture is quenched by adding to it excess water.

Following this direct methylation, attempts to convert unreacted lovastatin to the triol acid or the diol lactone for final product purification purposes were made using NaOH or LiOH. However, this base hydrolysis hydrolyzed only a small percentage of the lovastatin. Thus, unreacted lovastatin remained as a contaminant of the final simvastatin product. Furthermore, the base hydrolysis also hydrolyzed simvastatin, thus reducing yields of the desired simvastatin product. Following hydrolysis, the open ring acid form of simvastatin or a salt form thereof was then converted to the lactone by either heat or acid-catalyzed lactonization, and separated and purified by crystallization.

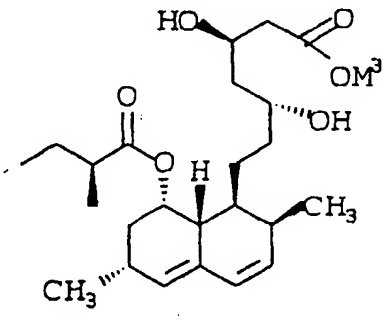
DETAILED DESCRIPTION OF THE INVENTION

The present invention is concerned with the purification and isolation of simvastatin from mixtures of simvastatin and lovastatin in high purity and yield employing a fungus or bacterium capable of selectively hydrolyzing the lovastatin 2-methylbutyryloxy side chain to 6(R)-[2-8(S)-hydroxy-2(S),6(R)-dimethyl-1',2',6',7',8',8a'(R)-hexahydronaphthyl) ethyl)-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one, the triol acid or the corresponding diol lactone. The triol acid or diol lactone is easily separable from the simvastatin (or simvastatin acid) by conventional means such as crystallization, high pressure liquid chromatography or other chromatographic methods. The present invention is particularly useful in removing unreacted lovastatin from simvastatin in the synthesis of simvastatin from lovastatin.

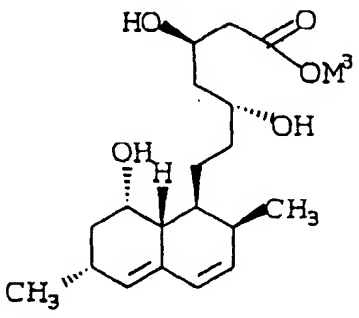
The process of the present invention may be used to separate mixtures of simvastatin and lovastatin either in their lactone forms or in their acid forms. Since the acid forms of lovastatin and simvastatin are more soluble in aqueous systems than the lactone form, the use of the acid form is preferred.

Typically the lovastatin and simvastatin will be employed in the salt form. Unless otherwise specified, the terms "acid", "open ring acid" and "acid form", when applied to the starting materials, intermediates and final products of the present invention include any suitable salt form thereof as well. Any salt which permits good solubility and which will not interfere with the other conditions encountered in carrying out the particular reaction is permissible. For example, the alkali metal salts, such as lithium, sodium and potassium; alkaline earth metal salts, such as calcium or magnesium; or salts with other metals such as aluminum, iron, zinc, copper, nickel or cobalt; amino acid salts formed from basic amino acids, such as arginine, lysine, α,β-diaminobutyric acid and ornithine; amine salts such as t-octylamine, dibenzylamine, ethylenediamine, morpholine, and tris(hydroxymethyl)aminomethane; or the ammonium salt may be employed. The alkali metal salts (Li, Na, and K) and the ammonium salt forms of the lovastatin acid may be employed and are preferred. Especially preferred are the potassium and ammonium salt forms.

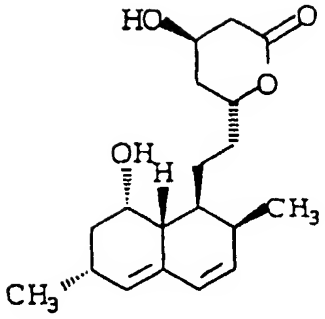
For convenience, the structural formulas for lovastatin acid, the triol acid, its lactone form, and simvastatin are set out below as Formulas 1, 2, 3, and 4 respectively:



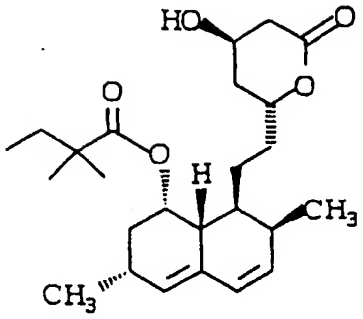
1 Lovastatin Acid



2 Triol Acid



3 Diol Lactone



4 Simvastatin

wherein:

M^3 is selected from the group consisting of

a) H,
b) an alkali metal salt such as Li, Na or K,
c) an alkaline earth metal salt such as Ca or Mg,
d) a salt with other metals such as Al, Fe, Zn, Cu, Ni or Co,
e) an amino acid salt formed from a basic amino acid such as arginine, lysine, α,β-diaminobutyric acid, or ornithine,
f) an amine salt such as t-octylamine, dibenzylamine, ethylenediamine, morpholine. or tris(hydroxy-methyl) aminomethane, and
g) the ammonium salt.

As already explained, for reasons of solubility, it has been found most desirable to use mixtures of lovastatin and simvastatin in their open ring or acid forms, and for this purpose the ammonium, potassium, sodium and lithium salt forms of lovastatin acid are preferred.

Fungi which are useful in the process of the present invention are those fungi which selectively cleave the lovastin 2-methylbutyryloxy side chain in the presence of simvastatin. Fungi of genera Clonostachys, Emericella, Diheterospora, Humicola, Dichotomonyces, Neocosmospora, Scopulariopsis, Xylogone, Torulomyces and Thievela are capable of hydrolyzing the lovastatin side chain. Particularly useful fungi include: Clonostachys compactiuscula, Monascus ruber, Mortierella isabellina, Emericella unguis, Diheterospora chlamydosporia, Humicola fuscoatra, Dichotomomyces cejpii, Neocosmospora africana, Xylogone sphaerospora, Torulomyces ragena, Thielavia fimeti, Aspergillus unguis, Mucor circinelloides, Fusarium solani, Penicillium chrysogenum, Aspergillus clavatus, Scopulariopsis communis, Gilmaniella humicola, Mucor bainieri, Tricharus spiralis, and Chaetomium cochliodes.

Especially preferred are the fungi Clonostachys compactiuscula, Humicola fuscoatra, Neocosmospora africana, Scopulariopsis communis, and Xylogone sphaerospora. The most preferred strain is Clonostachys compactiuscula (ATCC 74178 or ATCC 38009).

Bacteria which are useful in the process of the present invention are those bacteria which selectively cleave the lovastin 2-methylbutyryloxy side chain in the presence of simvastatin.

Actinomycetes of genera Streptomyces, Planomonospora and Kibdelosporangium are capable of hydrolyzing the lovastatin side chain. Particularly useful bacteria include: Streptomyces albogriscolus, Streptomyces paucisporogenes, Streptomyces hygroscopius, Streptomyces viridochromogenes, Planomonospora parontospora, and Kibdelosporangium aridum.

The mixture of lovastatin and simvastatin or the acids thereof may be treated with the microbe itself, or mutants thereof, or a cell-free extract derived therefrom, or a hydrolase purified from the cell free extract (or the spent, cell-free broth or cutures medium in which the fungus or bacterium was grown.

The term "mutant" refers to an organism in which some gene (or its regulatory region of DNA) within its genome is modified, leaving the gene or genes responsible for the organism's ability to hydrolyze lovastatin acid to the triol acid functional and heritable. Mutants within the scope of this invention have essentially the same characteristics as those of the parent strain, and are capable of hydrolyzing the lovastatin 2-methylbutyryloxy side chain.

The enzyme produced by the microbial culture or a mutant thereof may be brought into contact with the mixture of simvastatin and lovastatin in any number of ways, all of which will be apparent to the person of ordinary skill in this art. All of these are within the definition of the term "treating" as defined in this invention. For example, whole fermentation broth may be used, and in accordance with this procedure, a fermentation culture of the microbe is produced to which the mixture of simvastatin and lovastatin is simply added and the pure simvastatin product recovered.

A variation of this whole broth procedure is one in which a fermentation culture of the microbe as described above is produced, but a small concentration (0.5 to 2.5 g/L, preferably 1.0 to 2.0 g/L) of lovastatin acid is added for the purpose of inducing hydrolytic activity. The cell mass is then harvested by centrifugation or filtration and recovered as pellets or as a hyphal mat which can be used immediately or frozen for later use. The pellets or mat may then be added directly to the mixture of simvastatin and lovastatin resulting from conversion of lovastatin to simvastatin via methylation. Alternatively, the mixture of lovastatin and simvastatin may be partially purified and then brought into contact with the frozen pellets of the microbial culture described above.

It is not necessary that the whole cells of the fungus be alive. It is also possible to employ dead cells, e.g., those which have been acetone-dried.

As an alternative to whole cells, it is possible to use crude homogenates derived from these whole cell cultures. It is also possible to isolate the hydrolytic enzyme itself from the crude homogenates and employ the substantially purified hydrolytic enzyme.

When the microorganism excretes the hydrolytic enzyme into the fermentation/culture medium, it is possible to employ the isolated enzyme.

The process of bringing the hydrolytic enzyme into contact with the mixture of simvastatin and lovastatin starting material may be carried out batch-wise, or it may be carried out in a continuous manner. The contacting of these reactants themselves may be modified in various ways in keeping with advances in process technology. Thus, an immobilized enzyme column may be employed for the hydrolytic enzyme with the mixture of simvastatin and lovastatin being passed through the column. Another example of such process technology is that relating to membrane reactors. The preferred methods of contacting the reactants is by way of the immobilized enzyme column described above or by using a purified enzyme preparation.

Working examples set out further below describe the method currently employed to demonstrate the enzymatic hydrolysis of contaminating lovastatin to triol acid in the presence of simvastatin to simvastatin of high purity. However, the methods in those working examples would not necessarily be suggestive of methods which would be utilized for commercial production.

The use of the process of this invention to separate and purify simvastatin from mixtures of simvastatin and lovastatin is shown in Scheme II.

The mixture of the simvastatin and lovastatin lactones is converted to a mixture of the corresponding open-ring acids, preferably by treatment with an essentially stoichiometric aqueous alkali hydroxide such as potassium hydroxide or sodium hydroxide in a hydrocarbon solvent such as benzene, toluene or cyclohexane containing a small amount of a C_{1-3} alkanol, preferably isopropanol, ethanol or methanol, stirring for a few minutes to about an hour. The substrate is then extracted into an aqueous medium, such as TRIS (Tris(hydroxymethyl)-aminomethane), glycine, TES (N-tris [Hydroxymethyl)-methylamino]-2-hydroxy-propane-sulfonic acid), sodium phosphate, MOPSO (3-[N-Morpholino]-2-hydroxypropane-sulfonic acid), BIS-TRIS PROPANE (1,3-bis[tris-(Hydroxymethyl)methylamino]propane), BES (N, N-bis-[2-hydroxyethyl]-2-aminoethanesulfonic acid), MOPS (3-[N-Morpholino]-propanesulfonic acid), HEPES (N-[2-Hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid]), DIPSO (3-[N,N-bis(2-Hydroxyethyl)amino]-2-hydroxypropane- sulfonic acid), TAPSO (3-[N-tris-(Hydroxymethyl)-methylamino]-2-hydroxypropanesulfonic acid), HEPPSO (N-[2-Hydroxyethyl]piperazine-N'-[2-hydroxypropane- sulfonic acid]), POPSO (Piperazine-N,N'-bis[2-hydroxypropane sulfonic acid]), EPPS (N-[2-Hydroxyethyl]-piperazine-N'-[3-propanesulfonic acid], TEA (N-tris[Hydroxymethyl]methyl-2-aminoethane sulfonic acid), TRICINE (N-tris[Hydroxymethyl]-methyl-glycine), BICINE (N,N-bis[2-Hydroxyethyl]-glycine), TAPS (N-tris[Hydroxymethyl]methyl-3-aminopropane sulfonic acid), AMPSO (3-[(1,1-Dimethyl-2-hydroxyethyl) amine]-2-hydroxypropanesulfonic acid) or CHES (2-[N-Cyclohexylamino]-2-hydroxypropanesulfonic acid) buffer, pH 7-10, 25 mM to 1 M; distilled water, or one of the aqueous media listed above supplemented with up to 20% (vol./vol.) of a water-miscible solvent such as methanol, ethanol, propanol, butanol, or tetrahydrofuran. Preferred are TRIS, glycine, TES and sodium phosphate buffers, pH 7.5-9.5, 25 mM to 75 mM containing 12 % methanol. The dissolved or suspended substrate is then treated with the microbe or a mutant thereof or a cell-free extract derived therefrom or a hydrolase derived from the microbe or the substrate is converted to the ammonium salt and treated with the microbe or a mutant thereof or a cell-free extract derived therefrom or a hydrolase derived therefrom. The aqueous system may be added prior to or simultaneous with the addition of the selected microbe or acceptable mutants thereof, or the cell-free extract derived therefrom or the hydrolase.

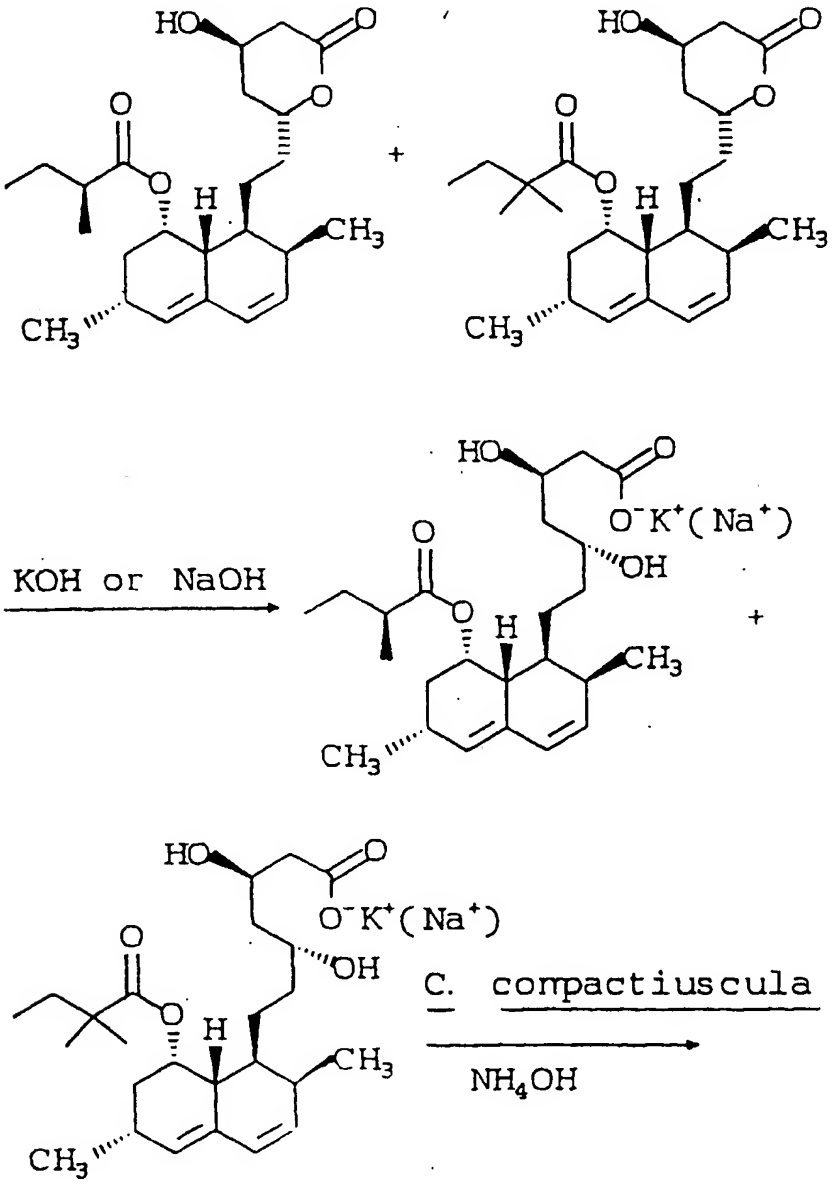
Lactonization by either acid-catalyzed or heat-catalyzed methods, for example, by stirring in isopropylacetate (IPAC) containing 7 mM methane sulfonic acid for two hours at room temperature follows. The resulting simvastatin lactone and diol lactone are separable by high pressure liquid chromatography (HPLC) or by crystallization to obtain

substantially pure simvastatin.

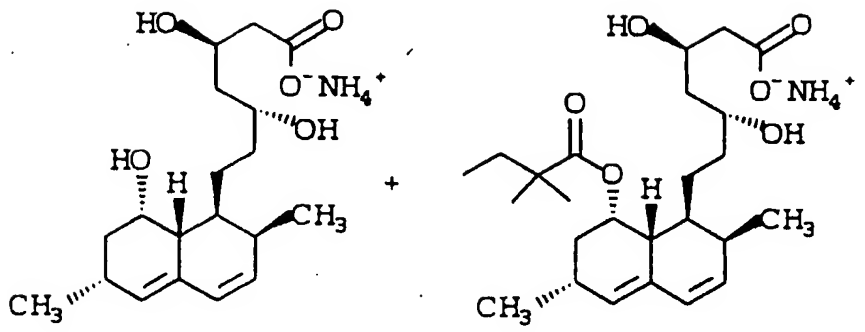
Reversed-phase HPLC is conducted using as a mobile phase an organic-aqueous mixture with the aqueous component being 0.01 to 1.0 % phosphoric acid or trifluoroacetic acid or other suitable acid and suitable organic components include acetonitrile, methanol and ethanol.

Simvastatin may also be isolated from the triol acid/diol lactone and purified by crystallization from ethyl acetate, isopropyl acetate and methanol.

SCHEME II

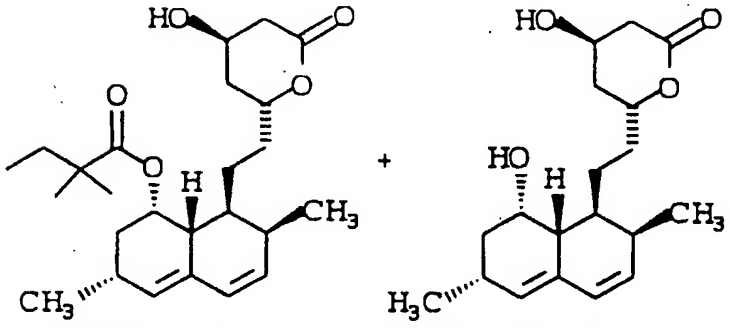


SCHEME II cont'd



methanesulfonic acid

IPAC



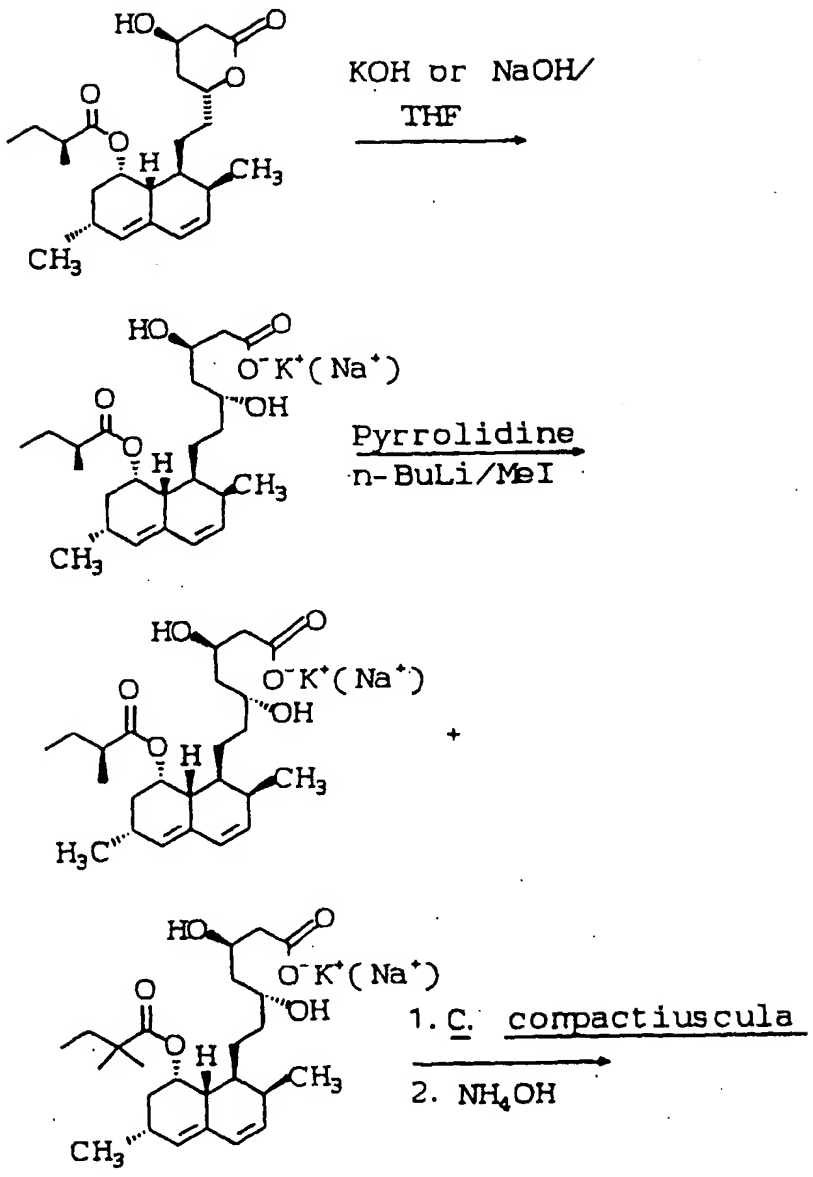
separable by crystallization, HPLC

The enzymatic hydrolysis of lovastatin acid to the triol acid can also be employed in the process for making simvastatin by direct methylation of lovastatin. This overall process is shown in Scheme III.

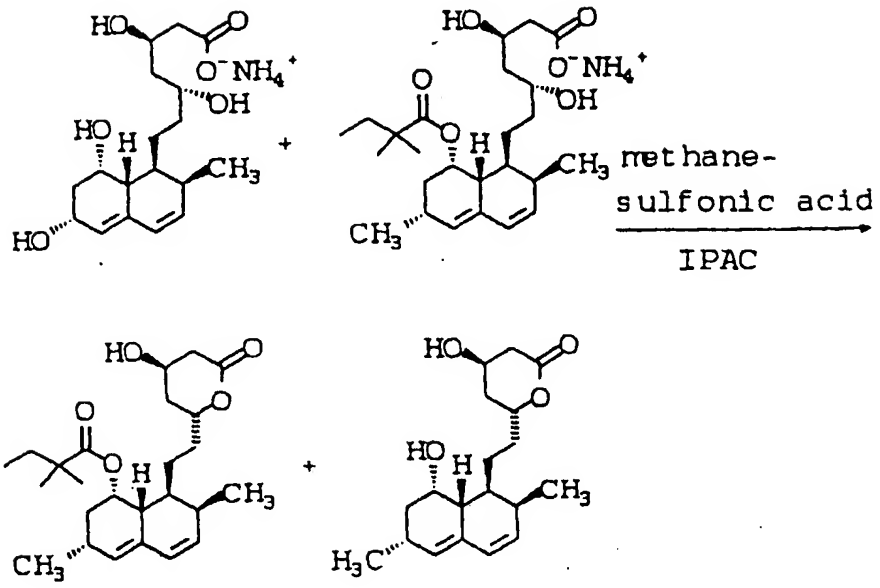
In the process of forming simvastatin by the direct methylation of lovastatin, the lovastatin lactone compound is first converted to an alkali metal salt, preferably potassium salt of the dihydroxycarboxylate. Although any conceivable method of preparing a dry salt would suffice, it is convenient to add a substantially stoichiometric amount of aqueous potassium hydroxide to a solution of the lactone starting material in a hydrocarbon solvent such as benzene, toluene or cyclohexane containing a small amount of a C_{1-3}alkanol, preferably isopropanol, ethanol or methanol, or alternatively employing tetrahydrofuran (THF), with or without the added alkanol, stirring for a few minutes to about an hour and finally concentrating to dryness in vacuo. The residue is subjected to rigorous water removal such as by azeotropic distillation with cyclohexane, toluene, or dry tetrahydrofuran, preferably extremely dry (less than 0.08 mg H_2O/mL) tetrahydrofuran.

The dry alkali metal salt is dissolved in an ethereal solvent such as tetrahydrofuran, diethyl ether, 1,2-dimethoxyethane or the like, cooled to about -80°C to -25°C, preferably -35°C to -30°C and treated with an excess of a strong base such as an alkali metal amide, wherein the alkali metal is lithium, sodium or potassium, preferably lithium, and the amide is diethylamide, pyrrolidide, dimethylamide or diisopropyl amide in an etheral solvent in a dry inert environment. After about 2 to 8 hours, preferably about two hours at -80° to -25°C, preferably -35° to -30°C. a methylhalide, such as methyl bromide, methyl chloride or methyl iodide, preferably methyl bromide or methyl iodide, is added to the mixture while maintaining the low temperature. Treatment with the strong base and

SCHEME III


KOH or NaOH/
THF
Pyrrolidine
n-BuLi/MeI
1. C. compactiuscula
2. NH4OH

SCHEME III cont'd



methyl halide as described can be repeated if appreciable amounts of starting material remain. After 0.5 to about 3 hours following final addition of methyl halide, the reaction mixture is quenched by adding to it excess water.

The mixture of lovastatin-acid salt and simvastatin acid salt is then, preferably, converted to the corresponding ammonium salt by ammonium hydroxide-methanol in ethyl acetate and following isolation of the ammonium salt (preferably by crystallization) and resuspension in an aqueous medium, treating with the selected microbe, or a mutant thereof or a hydrolase derived therefrom.

Alternatively the hydrolytic enzyme is added directly to the mixture of lovastatin salt and simvastatin salt following the removal of residual organics by distillation.

The resulting mixture of simvastatin acid and triol acid may be converted to the corresponding mixture of lactones by a suitable method, for example, heat-catalyzed or acid-catalyzed lactonization. Simvastatin is separable from the resulting mixture of simvastatin and diol lactone by HPLC or crystallization. Alternatively, the simvastatin acid may be separated from the triol acid by HPLC or crystallization, followed by conversion of the pure simvastatin acid to simvastatin lactone. If the simvastatin acid is to be isolated and purified by crystallization, it is preferred to convert the simvastatin acid to the ammonium salt prior to lactonization.

There is also disclosed mutants of the particular strain of the microbial culture: Clonostachys compactiuscula (ATCC 38009 and ATCC 74178), Monascus ruber (FERM-P. No. 4822), Mortierella isabellina (IFO 7844, ATCC 42613, ATCC 36670, ATCC 38063, or ATCC 44853), Emericella unguis (IFO 8087, ATCC 10073, ATCC 12063, ATCC 13431, or ATCC 16812), Diheterospora chlamydosporia (IFO 9249, ATCC 16449, ATCC 18956, ATCC 20537), Humicola fuscoatra,(IFO 9530, ATCC 12774, ATCC 52073, ATCC 62175), Dichotomomyces cejpii (IFO 9929, ATCC 22149, ATCC 42284), Neocosmospora africana (IFO 7590, ATCC 24342), Xylogone sphaerospora (IFO 9516, ATCC 42027), Torulomyces ragena (IFO 30008), Thielavia fimeti (IFO 30419), Aspergillus unguis (MF 1416), Mucor circinelloides (ATCC 1207a), Fusarium solani (ATCC 12826), Penicillium chrysogenum (ATCC 10002), Aspergillus clavatus (ATCC 1007), Scopulariopsis communis (MF 3769), Gilmaniella humicola (ATCC 16013), Mucor bainieri (ATCC 42642), Tricharus spiralis (MF 5295), Chaetomium cochliodes (ATCC 10195), Streptomyces albogriscolus (NRRL 5748), Streptomyces paucisporogenes (ATCC 25482), Streptomyces hygroscopius (ATCC 21722), Streptomyces viridochromogenes (ATCC 21724), Planomonospora parontospora (ATCC 23864), and Kibdelosporangium aridum (NRRL 12647) which are capable of converting lovastatin acid to triol acid. There are techniques well known in the fermentation art for improving

the yields of desired products produced by various strains of microorganisms. For example, a given producing strain may be irradiated or exposed to other stimuli known to greatly increase the ongoing mutation of the genetic material of the microorganism. By using a sensitive screen, it is then possible to select from the many mutations thus produced only those which result in an enhanced production of the desired product. In this way, it is usually possible to continually improve the output of a producing strain through its various selected descendants. A biologically pure culture of a mutant is a culture that consists substantially of one strain of mutant. With regard to the present invention, similar improvements in output of lovastatin acid hydrolase by selected mutants of the fungi or actinomycetes may be achieved. A satisfactory screen for this purpose is the use of high performance liquid chromatography (HPLC) which can detect the enzymatic cleavage products at very low concentrations, thus clearly establishing the lovastatin has been converted to triol acid by any particular mutant in question.

Culture Medium

Fermentation of the microbes is carried out in aqueous media such as those employed for the production of other fermentation products. Such media contain sources of carbon, nitrogen and inorganic salts assimilable by the microorganism.

In general, carbohydrates such as sugars, for example, lactose, glucose, fructose, maltose, mannose, sucrose, xylose, mannitol and the like and starches such as grains, for example, oats, ryes, cornstarch, millet, corn meal and the like can be used either alone or in combination as sources of assimilable carbon in the nutrient medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 1% and 6% by weight of the medium. These carbon sources can be used individually, or several such carbon sources may be combined in the medium. In general many proteinaceous materials may be used as nitrogen sources in the fermentation process. Suitable nitrogen sources include for example, yeast hydrolysates, primary yeast, soybean meal, cottonseed flour, hydrolysates of casein, corn steep liquor, distiller's solubles or tomato paste and the like. The sources of nitrogen either alone or in combination, are used in amounts ranging from about 0.2% to 6% by weight of the aqueous medium.

Among the nutrient inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, ammonium, calcium, phosphate, sulfate, chloride, carbonate, and like ions. Also included are trace metals such as cobalt, manganese, iron and magnesium. In addition, if necessary, a defoaming agent such as polyethylene glycol or silicone may be added, especially if the culture medium foams excessively.

It should be noted that the media described in the Examples are merely illustrative of the wide variety of media which may be employed, and are not intended to be limitative. Specifically, the carbon sources used in the culture media include dextrose, dextrin, oat flour, oatmeal, molasses, citrate, soybean oil, glycerol, malt extract, cod liver oil, starch, ethanol, figs, sodium ascorbate and lard oil. Included as nitrogen sources were peptonized milk, autolyzed yeast, yeast RNA, tomato paste, casein, primary yeast, peanut meal, distillers solubles, corn steep liquor, soybean meal, corn meal, NZ amine, bean extract, aspargine, cottonseed meal and ammonium sulfate. The major ionic components are $CaCO_3$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and NaCl and small amounts of $CoCl_2 \cdot 6H_2O$ and traces of Fe, Mn, Mo, B, Co and Cu were also present.

Lactonization

Treatment of a mixture of simvastatin acid lovastatin acid with the microbial cultures capable of hydrolyzing the lovastatin 2-methylbutyryloxy side chain, or mutants thereof, or a cell-free extract derived therefrom, or a hydrolase derived therefrom, in accordance with the process of the present invention provides the easily separable mixture of simvastatin acid and triol acid. If the lactone form of simvastatin is desired, the product mixture may be lactonized and separated, the diol lactone separated from the simvastatin lactone, alternatively, the simvastatin acid may be separated from the triol acid, followed by lactonization of simvastatin acid to simvastatin. Lactonization of triol acid is carried out using standard procedures, i.e., either heat or acid catalyzed lactonization. Procedures for acid-catalyzed lactonization of lovastatin acid-related compounds are known and described in U.S. Patent 4,916,239. For simvastatin acid and the triol acid, lactonization has been carried out by stirring in isopropyl acetate containing 7 mM methane sulfonic acid for 2 hours at room temperature.

EXAMPLE 1

Biotransformation of lovastatin acid to triol acid by whole cells of Clonostachys compactiuscula

Clonostachys compactiuscula ATCC 38009 was grown in a 2 L airlift fermentor with 1.8 L working volume in medium EN (glucose 1%; peptone 0.2%; beef extract 0.1%; yeast extract 0.1%; and corn steep liquor 0.3%), at 29°C, at an

aeration rate of 1.25 vvm, for 48-72 hrs. Lovastatin ammonium salt was added (0.5 g/L final concentration) to induce hydrolytic activity. The fermentation was harvested 24-72 hrs. after addition of the lovastatin ammonium salt by straining through a sieve and washing the pellets with buffer (20 mM Tris, pH 8.5). The cell pellets were frozen until ready to use.

For the biotransformation, Clonostachys compactiuscula pellets (17 g wet weight) from an airlift fermentation were contacted with 20 mL crude lovastatin acid (@20 g/L) in carbonate buffer harvested from an Aspergillus terreus fermentation. The biotransformation was carried out in a 250 mL Erlenmeyer flask at 27°C and 160 rpm. After 17 hrs. approximately 60% of the lovastatin acid was converted to triol acid.

In an additional experiment, Clonostachys compactiuscula pellets from an airlift fermentation (5 g wet weight) were contacted with 10 mL crude lovastatin acid (3.5 g/L) extracted from an Aspergillus terreus fermentation by methanol. The final concentration of methanol in the biotransformation mixture was 25%. The bioreaction was carried out in a 250 mL Erlenmeyer flask at 27°C and 160 rpm. After 2 hrs. the biotransformation employing Clonostachys compactiuscula converted nearly 100% of the lovastatin acid to triol acid, as measured by thin layer chromatography.

EXAMPLE 2

Biotransformation of lovastatin acid to triol acid by crude homogenate of Clonostachys compactiuscula

Clonostachys compactiuscula ATCC 38009 was grown in 250 mL shake flasks containing 12 mL of medium EN at 29° C for 3 days. Lovastatin ammonium salt was added to give a concentration of 2.5 g/L and fermentation was continued for 2 additional days. To prepare the crude homogenate, the culture was harvested by centrifugation at 3000 rpm for 10 minutes, after which it was washed with 50 mM of N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer, pH 7.7. The culture medium was again centrifuged and the cell mass was chilled on ice and then subjected to grinding in a mortar and pestle containing glass fragments and powdered dry ice. Ground homogenate equivalent to the contents of 1 shake flask was resuspended in 2.0 mL of 50 mM TES buffer and centrifuged at 6000 rpm for 10 minutes to remove cell debris and glass fragments. The supernatant was used as the source of crude homogenate with a protein concentration of approximately 0.5 mg/mL.

In order to carry out the biotransformation, one volume of crude homogenate was combined with an equal volume of lovastatin acid ammonium salt (5 g/L), and the mixture was incubated at 29° C. Using this method, 80-90% conversion of lovastatin acid to triol acid was observed within 2 hrs.

EXAMPLE 3

Purification of the lovastatin hydrolyzing enzyme from Clonastachys compactiuscula cells

A hydrolytic enzyme which carries out the biotransformation of lovastatin acid to triol acid was purified by Fast Protein Liquid Chromatography (FPLC*) employing a MONO Q® anion exchange column to near homogeneity from homogenates of Clonostachys compactiuscula employing the procedures described below.

The supernatant from the 6,000 rpm centrifugation as in Example 2 above, but where 50 mM of tris(hydroxymethyl) aminomethane (TRIS) buffer (pH 7.8) is substituted for 50 mM TES buffer, was centrifuged at 15,000 rpm for 20 minutes and the resulting supernatant filtered through a 0.45 mm filter. Batches (10 mL) of filtrate containing 0.3-0.5 mg/mL protein were then applied at a rate of 1.0-2.0 mL/minute to a Pharmacia MONO Q® (HR 5/5) anion exchange column connected to a Pharmacia Fast Protein Liquid Chromatography (FPLC) system.

After allowing binding of the anionic proteins to the column matrix, the hydrolase was specifically eluted by the application of a linear gradient of sodium chloride (0-500mM) in 20 mM TRIS, pH 7.8. Eluted protein was collected in 1 mL fractions and assayed either using lovastatin ammonium salt (in which case percent hydrolysis was estimated by TLC (thin-layer chromatography) and densitometry or HPLC), or a colorimetric substrate (ortho-nitrophenyl butyrate, o-NPB) towards which the enzyme had been shown to have hydrolytic activity. When the latter substrate was used, the hydrolytic reaction was monitored spectrophotometrically at 410 nm essentially as described by Lawrence, R.C. et al. in J. Gen. Microbiol. (1967) 48, 401-418. Both assay methods revealed that the hydrolase was eluted when the NaCl concentration approached 300 mM.

Sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis revealed the peak lovastatin acid hydrolase-containing fractions to contain a prominent band of molecular weight approximately 45,000 Da.

Using the purified enzyme preparation, the biotransformation was carried out in accordance with the procedures described above in Examples 1, 2, 4 and 6, and an estimate was made of the hydrolase's Km and specific activity with lovastatin ammonium salt as substrate. The value for Km obtained was 4.14 mM and under saturating substrate conditions the enzyme was found to have a specific activity of 0.04 mmol lovastatin ammonium salt hydrolyzed/mg protein per minute.

EXAMPLE 4

Biotransformation of lovastatin acid to triol acid by purified hydrolase from Clonostachys compactiuscula

A hydrolytic enzyme which carries out the biotransformation of lovastatin acid to triol acid was purified by Fast Protein Liquid Chromatography (FPLC*) employing a MONO Q® anion exchange column to near homogeneity from homogenates of Clonostachys compactiuscula employing the procedures described below.

A supernatant from the 6,000 rpm centrifugation as in Example 2 above, but where 20 mM of tris(hydroxymethyl) aminomethane (TRIS) buffer is substituted for 50 mM TES buffer, was centrifuged at 15,000 rpm and the resulting supernatant filtered through a 0.45 micrometer filter. Batches (10 mL) of filtrate containing 0.3-0.5 mg/mL protein were then applied to a Pharmacia MONO Q® anion exchange column connected to a Pharmacia Fast Protein Liquid Chromatography (FPLC) system.

After allowing binding of the anionic proteins to the column matrix, the hydrolase was specifically eluted by the application of a linear gradient of sodium chloride (0-500 mM). Eluted protein was collected in 1 mL fractions and assayed either using lovastatin ammonium salt (in which case percent hydrolysis was estimated by TLC and densitometry or HPLC), or a colorimetric substrate (ortho-nitrophenyl butyrate o-NPB) towards which the enzyme had been shown to have hydrolytic activity. When the latter substrate was used, the hydrolytic reaction was monitored spectrophotometrically at 410 nm essentially as described by Lawrence, R.C. et al. in J. Gen. Microbiol. (1967) 48, 401-418. Both assay methods revealed that the hydrolase was eluted when the NaCl concentration approached 300 mM.

Sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis revealed the peak lovastatin acid hydrolase-containing fractions to contain a prominant band of molecular weight approximately 45,000 Da.

Using the purified enzyme preparation, the biotransformation was carried out in accordance with the procedures described above in Examples 1 and 2, and an estimate was made of the hydrolase's Km and specific activity with lovastatin ammonium salt as substrate. The value for Km obtained was 4.14 mM and under saturating substrate conditions the enzyme was found to have a specific activity of 110 umol lovastatin ammonium salt/mg protein per hour.

EXAMPLE 5

Biotransformation of lovastatin ammonium salt in the presence of excess simvastatin ammonium salt.

Forty-five grams of frozen Clonostachys compatiuscula (ATCC 38009) cells, which had been grown in medium EN as detailed in Example 2 (and washed with 50 mM Tris buffer, pH 7.8, prior to freezing) was homogenized with glass fragments and dry ice using a mortar and pestle. The resulting homogenized, frozen powder was transferred to a suitable tube and the material remaining in the mortar washed into the same tube using a minimal volume of 50 mM Tris, pH 7.8. The mixture was then allowed to thaw and then centrifuged at 6000 rpm for 10 minutes to remove large cell debris and glass.

The 6000 rpm supernatant was used as a crude source of hydrolase and 0.8 mL was mixed with 0.2 mL methanol and 1.0 mL of a solution of simvastatin (18.6 mM and lovastatin (1.4 mM) ammonium salts in 50 MM Tris, pH 7.8.) The reaction mixture was incubated at 29 C and sampled after lh, 2h, and 17h by removing 0.1 mL and diluting with 0.9 mL methanol. The samples were then subjected to analysis by HPLC using a Whatman C-8 column as stationary phase and a 60:40 mixture of acetonitrile: 0.5% phosphoric acid as mobile phase; under these conditions the respective retention times for simvastatin, lovastatin and triol ammonium salts are 4.4 min., 3.8 min., and 2.5 min. After 17h the area percent of the lovastatin peak had been reduced from 23.2% to 0.7%, representing a greater than 99% conversion. Greater than 96% of the initial simvastatin ammonium salt remained intact over this same contact period.

EXAMPLE 6

Biotransformation of residual lovastatin acid to triol acid following the synthesis of simvastatin acid from lovastatin acid by direct methylation.

Step 1: Preparation of Lovastatin Potassium Salt

A solution of lovastatin (99% pure; 25 g; 60.57 mmol) in 325 mL tetrahydrofuran (THF) was prepared under nitrogen then cooled to 5°C. An aqueous solution (6.1 mL) of 10.01 M potassium hydroxide was added over 15 min then the mixture was warmed to 25°C and aged, with stirring, until complete (>99%) conversion to the potassium salt (by HPLC analysis) had occurred.

Step 2: Preparation of Simvastatin Potassium Salt

The lovastatin potassium salt solution prepared in Step 1 was heated to reflux, distilling a total of 500-700 mL THF through a 10 in. Vigreaux column while maintaining a minimum pot volume of 215 mL with sieve-dried THF. The water content of the lovastatin potassium salt solution was thus reduced to a level of <0.1 mg/mL. This solution was then diluted with 150 mL of sieve-dried THF (water content <0.1 mg water/mL) to give a total volume of 365 mL. Sieve-dried pyrrolidine (5.81 g; 81.7 mmol; water content <0.2 mg/mL) was added as a single batch and the reaction cooled in a dry ice/acetone bath to -78°C. Next, 117 mL of 1.6 M n-butyllithium in hexane was added over a one hour period, sub-surface, while maintaining rapid agitation and an internal temperature below -70°C.

The lovastatin potassium salt solution, now containing the lithium pyrrolidide intermediate, was warmed to -35°C using a dry ice-acetonitrile bath and aged for 2 hours. After recooling to -45°C, 13.32 g of sieve-dried methyl iodide (93.0 mmol; density 2.89 g/mL) was added in one portion and the mixture aged at -30°C (internal temperature following methyl iodide addition) for 30 minutes. The mixture was quenched with 200 mL water and the phases allowed to separate in a separating funnel. The lower, aqueous, layer was diluted to a volume of 1250 mL by the further addition of water and then cooled to below 10°C. The pH was adjusted to 6 using 6 M hydrochloric acid then 250 mL ethyl acetate was added and the pH further adjusted to 2.0 (again using HCl). Phase separation was again allowed to occur then the aqueous layer was re-extracted with 175 mL cold (5-10°C) ethyl acetate. The two organic (ethyl acetate) layers were pooled and then washed with 150 mL water before drying the final organic layer over sodium sulfate (to <10 mg/mL water) and filtering. Next, 112.3 mL methanol was charged into the (425 mL) dry, filtered mixture at 25°C and then 1.3 mL of a methanol:aqueous ammonium hydroxide (3:1) solution was added over a 5 minute period. The mixture was seeded with simvastatin ammonium salt (SAS) and aged for 10 minutes then a further 35.9 mL of the methanol:aqueous ammonium hydroxide (3:1) solution was added dropwise over 1 hour. The mixture was then cooled to -10°C over 2.5 hours and aged for an additional 1 hour. The product was filtered and washed with 25 mL cold (0°C) methanol and the resulting white crystals were dried in vacuo to give simvastatin ammonium salt as white needles (87% pure SAS containing 10% residual lovastatin as the ammonium salt).

Step 3: Biotransformation of residual lovastatin acid (as the ammonium salt) to triol acid

Clonostachys compactiuscula esterase was purified from 57 g mycelial cells which had been grown up in medium EN using the methods detailed in Examples 1 and 3. The use of a Pharmacia HR 10/10 MONO Q® column allowed the application of 85 mL of crude cell-free extract per purification run. In total 0.89 mg of purified esterase was obtained (in a volume of 10 mL) which was then concentrated to 0.175 mg protein/mL by ultrafiltration using a 10,000 molecular weight cut-off CENTRIPREP® device (AMICON®).

Samples of the esterase were then incubated with the simvastatin ammonium salt prepared by direct methylation of lovastatin; final concentrations of protein were 0.4, 4.0 and 40 microgram/mL and simvastatin concentrations used were 10, 35 and 50 mM. Other conditions which were varied were pH (7.8 and 9.5 were assessed) and methanol concentration (0, 10 and 20% [v/v, final concentration]). The reactions were buffered by the inclusion of either 100 mM TRIS (in the case of reactions carried out at pH 7.8) or 100 mM glycine (pH 9.0). Greater than 90% hydrolysis of residual lovastatin acid to triol acid was obtained within 16 h under the following conditions:

Enzyme conc.	Simvastatin conc.	pH	Methanol conc.
(microgram/ml)	(mM)		(% v/v)
4.0	10	7.8	0
4.0	10	7.8	10
4.0	10	9.5	0
4.0	10	9.5	10
4.0	10	9.5	20
4.0	35	9.5	10
40.0	35	7.8	0
40.0	35	7.8	10
40.0	35	9.5	0
40.0	35	9.5	10
40.0	35	9.5	20

EXAMPLE 7

Biotransformation of residual lovastatin acid to triol acid following the synthesis of simvastatin acid from lovastatin acid by direct methylation.

Step 1: Preparation of Simvastatin Ammonium Salt

Starting with 5 g lovastatin, the potassium salt solution in THF is prepared according to Example 6, Step 1. A solution of sieve-dried pyrrolidine (2.48 mL; 2.4 equivalents; 29.67 mmoL; <0.2 mg water/ml) in 12.3 mL sieve-dried THF) is cooled to -20°C in a dry ice/acetonitrile bath. Then a solution of 1.6M butyllithium in hexane (18.2 mL; 2.35 equivalents) is added at such a rate as to keep the temperature below -10°C. After the addition is complete the lithium pyrrolidide/THF solution is aged at -20°C for 15 minutes. The dry solution of lovastatin potassium salt in THF is cooled to -35°C in a dry ice/acetonitrile cooling bath. The lithium pyrrolidide/THF solution at -20°C is added to the rapidly agitated mixture at such a rate as to maintain the internal temperature below -30°C at all times throughout the addition. The mixture is aged at -35°C for 2 hours then, following cooling to -40°C, 1.16 mL (18.67 mmol; 1.5 equivalents) methyl iodide is added to the solution in a single batch which causes the internal temperature of the mixture to rise (to approximately -20°C); the internal temperature is brought back to -30°C and aged for 1 hour, then warmed to -10°C and aged for 30 minutes.

The mixture is quenched with 40 mL water and the phases allowed to separate in a separating funnel. The lower, aqueous, layer is diluted to a volume of 250 mL by the further addition of water and then is cooled to below 10°C. The pH is adjusted to 6 using 6 M aqueous hydrochloric acid then 50 mL ethyl . acetate is added and the pH further adjusted to 2.0 (again using HCl). Phase separation is again allowed to occur then the aqueous layer was re-extracted with 35 mL cold (5-10°C) ethyl acetate. The two organic (ethyl acetate) layers are pooled and then washed with 30 mL water before drying the final organic layer over sodium sulfate and filtering. Next, 22.5 mL methanol is charged into the dry, filtered mixture at 25°C and then 0.26 mL of a methanol:aqueous ammonium hydroxide (3:1) solution is added over 5 minutes. The mixture is seeded with simvastatin ammonium salt and aged for 10 minutes then a further 7.2 mL of the methanol/ammonium hydroxide is added dropwise over 1 hour. The mixture is then cooled to -10°C over 2.5 hours and aged for an additional 1 hour. The product is filtered and washed with 5 mL cold (0°C) methanol and the resulting white crystals are dried in vacuo to give simvastatin ammonium salt .

Step 2: Biotransformation of residual lovastatin acid (as the ammonium salt) to triol acid

Biotransformation is conducted according to the procedures in Example 6, Step 3.

EXAMPLE 8

Lactonization of Simvastatin Ammonium Salt and Crystallization and Isolation of Pure Simvastatin Lactone

Step 1: Lactonization of Simvastatin Ammonium Salt

Distilled water (20 mL) glacial acetic acid (40 mL) and butylated hydroxyanisole (BHA, 50 mg) were charged to a 250 ML 3-neck round bottom flask under a nitrogen atmosphere. The batch temperature was adjusted to 20-25°C and simvastatin ammonium salt (12.5 g, 27.56 mmoles) was added and agitated at 20-25°C for 15 min. or until dissolved. Methane sulfonic acid (70%, 4.35 g, 30.8 mmoles, 1.118 equiv) was added and the mixture was aged at 20-25°C for 2 hours until the lactonization reaction was more than 75% complete.

Percent conversion was determined by HPLC following the conditions in Preparation A. Percent conversion was calculated as follows:

$$\frac{\text{area \%(Simvastatin Ammonium Salt)}}{\text{area \%(Simvastatin Ammonium Salt + Simvastatin)}} \times 100\%$$

Step 2: Crystallization and Isolation of Pure Simvastatin

The batch was seeded with crude Simvastatin seed crystals (60 mg) and aged at 20-25°C for 0.5 hour. Distilled water (22.5 mL) was added over 3 hours (0.13 mL/min.) and a second distilled water charge (35 mL was added over one hour (0.58 mL/min.). The batch was aged at 20-25°C for one hour and then treated dropwise with 28 w/w% ammonium hydroxide (4.0 mL).

The batch was aged at 20-25°C for one hour and filtered to collect the Simvastatin crude crystals. The Simvastatin

crude wet cake was washed with 2:1) distilled water:acetic acid (50 mL), distilled water (50 mL) and 1:1 methanol: distilled water (50 mL). The product was dried overnight in vacuo with a nitrogen purge at 25-30°C to give the Simvastatin crude as white needles (10.38 g HPLC assay 98 w/w%).

EXAMPLE 9

Crystallization and Isolation of Pure Simvastatin

Crude Simvastatin (10 g, 23,89 mmoles) and butylated hydroxyanisole (50 mg) were charged to a flask containing 126.4 mL degassed methanol under a nitrogen atmosphere. The batch temperature was adjusted to 20-25°C and agitated for 15 minutes until solids dissolved. The solution was filtered through a bed of ECOSORB C® which is activated carbon composed of: water, activated carbon, cellulose fiber, styrene divinyl benzene and anion exchange resin (91.5 g of methanol (50 mL) washed ECOSORB C®) and the carbon cake is washed with 40 mL of degassed methanol. The combined methanol solution was transferred to a 250 mL 3 neck round bottom flask and heated to 38-40°C under a nitrogen atmosphere. Degassed distilled water (83.3 mL) was added subsurface over 30 minutes (2.78 mL/min.) and aged at 38-40°C for 30 minutes. The batch was cooled to 25°C over 1 hour. Degassed distilled water (83.3 mL was charged subsurface over 1 h (1.38 mL/min.) at 25°C and cooled to 10-15°C over 1 hour.

The slurry was filtered and the wet cake was washed with 50 mL of 50% methanol/distilled water (vol./vol.) at 10°C. The product was dried overnight in vacuo with a nitrogen purge at 35-40°C to give pure simvastatin as white needles (9.49 g HPLC assay = 99 w/w%).

EXAMPLE 10

Screening of Fungal Microorganisms for Lovastatin Esterase Activity

The strains listed below in Table I were grown in 10 mL medium EN for 48 or 72 hours before adding to the flasks lovastatin (for the screens reported in column 2) or simvastin (for the screens reported in column 3) as their ammonium salts, to a final concentration of 2.5 g/L. The cultures were allowed to incubate for a further 96 hours before analyzing the broths for conversion of the lovastatin or simvastatin to triol acid by thin layer chromatography. The extent of hydrolysis was quantified through densitometric scanning of TLC plates and comparison to lanes run with standard concentrations of pure samples of Lovastatin ammonium Salt, Simvastatin Ammonium Salt and Triol Acid Ammonium Salt.

TABLE I

strain name	ATCC No.	percent lovastatin hydrolysis	percent simvastatin hydrolysis
Mortierella isabellina	42013	11	<1
Humicola fuscoatra		12	3
Aspergillus unguis		21	0
Mucor circinelloides	1207a	2	0
Fusarium solani	12826	9	0
Dichotomomyces cejpii	22149	2	0
Dichotomomyces cejpii	42284	4	0
Diheterospora chlamydosporia	16449	11	2
Diheterospora chlamydosporia	18056	12	4
Diheterospora chlamydosporia	20537	3	10
Emericella unguis	10073	9	2
Emericella unguis	12063	4	1
Emericella unguis	13431	4	0
Emericella unguis	16812	1	2
Humicola fuscoatra	12774	57	0
Humicola fuscoatra	52037	14	4
Humicola fuscoatra	62175	25	7
Mortierella isabellina	36670	5	10

TABLE I (continued)

strain name	ATCC No.	percent lovastatin hydrolysis	percent simvastatin hydrolysis
Mortierella isabellina	38063	14	0
Mortierella isabellina	44853	12	6
Neocosmospora africana	24342	73	5
Xylogone sphaerospora	42047	48	8
Penicillium chrysogenum	10002	9	0
Aspergillus clavatus	1007	7	0
Scopulariopsis communis		30	0
Gilmaniella humicola	16013	5	0
Mucor bainieri	42642	1	0
Tricharus spiralis		2	0
Chaetomium cochliodes	10195	6	0
Scopulariopsis communis		40	0
Clonostachys compactiuscula		100	5
	38009		
	74178		
Clonostachys compactiuscula		87	3
	38009		
	74178		

EXAMPLE 11

Screening of Actinomycetes for Lovastatin Esterase Activity

The strains listed below in Table II were grown in 10 mL YN broth (1% meat extract, 0.5% yeast extract, 0.5% glucose, 0.6% peptone, pH 7.2) or 10 ML of ISP-1 medium for 72 hours before adding to the flasks lovastatin (for the screens reported in column 2) or simvastin (for the screens reported in column 3 as their ammonium salts, to a final concentration of 2.5 g/L. The cultures were allowed to incubate for a further 96 hours before analyzing the broths for conversion of the lovastatin or simvastatin to triol acid by thin layer chromatography. The extent of hydrolysis was quantified through densitometric scanning of TLC plates.

TABLE II

strain name	ATCC No.	percent lovastatin hydrolysis	percent simvastatin hydrolysis
Streptomyces albogriscolus		6	3
*NRRL No.	5748		
Streptomyces paucisporogenes		13	0
	25482		
Streptomyces hygroscopius		25	5
	21722		
Streptomyces viridochromogenes		7	0
	21724		
Planomonospora parontospora		7	0
	23864	2	0
Kibdelosporangium aridum		18	0
*NRRL No.	12647		

PREPARATION A

HPLC Weight Percent Assay for Dry Simvastatin Crude

30 mg of standard or sample were accurately weighed into a 100 mL volumetric flask and were diluted to the mark with 60:40 acetonitrile: 0.01 M KH_2PO_4 (pH = 4.0).

Column: PERKIN-ELMER® C_{18}, 3 cm length, 3 micron particle size, reversed-phase column

Temperature	25°C
Flow rate	3.0 mL/min
Detection	uv 238 nm
Injection	5 microliters
Mobile phase	50:50 acetonitrile: 0.1% H_3PO_4 (aq)

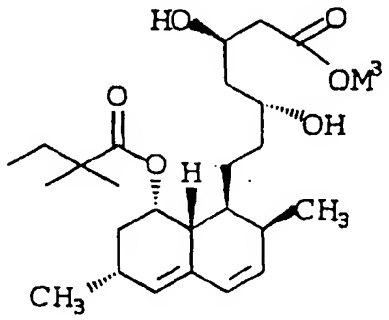
Retention Time:	
Time (min)	Identity
1.80	1. Simvastatin ammonium salt
2.20	2. Lovastatin and epimer
3.44	3. Simvastatin crude

The weight % is calculated as follows:

$$\frac{\text{(average response factor of samples) (100)}}{\text{(average response factor of standard)}} = \text{WEIGHT \%}$$

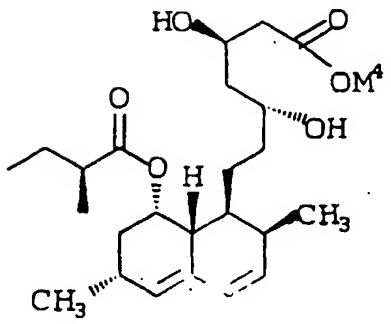
Claims

1. A process for separating a compound of Formula (4)



(4) Simvastatin Acid/Salt

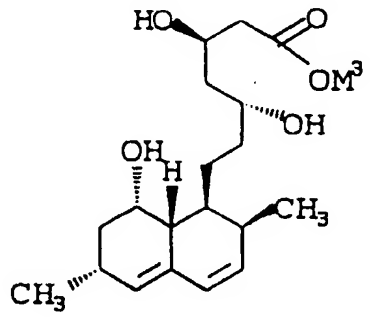
from a mixture thereof with a contaminant of Formula (1)



(1) Lovastatin Acid/Salt

comprising:

treating the mixture of the compounds with a microbial culture capable of selectively cleaving the lovastatin 2-methylbutyryloxy side chain, or with a hydrolase derived from the microbial culture to convert the compound of Formula (1) to the compound of Formula (2),



(2) Triol Acid/Salt

and separating and isolating the compounds of Formula (4) and Formula (2) in the open acid, salt or lactone form, wherein:

M^3 and M^4 are independently:

(a) H,
(b) Li, Na or K,
(c) Ca or Mg,
(d) Al, Fe, Zn, Cu, Ni, or Co,
(e) arginine, lysine, α,β-diaminobutyric acid, or ornithine,
(f) t-octylamine, dibenzylamine, ethylenediamine, morpholine, or tris(hydroxy-methyl)aminomethane, or
g) NH_4.

2. The process according to Claim 1 wherein the microbial culture is a fungal culture.

3. The process according to Claim 2 wherein the fungus is selected from the genera

(a) Clonostachys,
(b) Emericella,

(c) Diheterospora,
(d) Humicola,
(e) Dichotomonyces,
(f) Neocosmospora,
(g) Scopulariopsis,
(h) Xylogone,
(i) Torulomyces, and
(j) Thievela.

4. The process of Claim 3 wherein the fungus is:

(a) Clonostachys compactiuscula,
(b) Monascus ruber,
(c) Mortierella isabellina,
(d) Emericella unguis,
(e) Diheterospora chlamydosporia,
(f) Humicola fuscoatra,
(g) Dichotomomyces cejpii,
(h) Neocosmospora africana,
(i) Xylogone sphaerospora,
(j) Torulomyces ragena,
(k) Thielavia fimeti,
(l) Aspergillus unguis,
(m) Mucor circinelloides,
(n) Fusarium solani,
(o) Penicillium chrysogenum,
(p) Aspergillus clavatus,
(q) Scopulariopsis communis,
(r) Gilmaniella humicola,
(s) Mucor bainieri,
(t) Tricharus spiralis, or
(u) Chaetomium cochliodes.

5. The process according to Claim 4 wherein the fungus is:

(a) Clonostachys compactiuscula,
(b) Humicola fuscoatra,
(c) Neocosmospora africana,
(d) Xylogone sphaerospora, or
(e) Scopulariopsis communis.

6. The process according to Claim 5 wherein the fungus is Clonostachys compactiuscula (ATCC 74178 or 38009) or a mutant thereof.

7. The process according to Claim 4 wherein the mixture is treated with a purified form of the hydrolase derived from the culture therein.

8. The process according to Claim 1 wherein the- microbial culture is a bacterial culture.

9. The process according to Claim 8 wherein the bacterium is an actinomycete.

10. The process according to Claim 9 wherein the actinomycete is selected from the genera

(a) Streptomyces,
(b) Planomonospora, and
(c) Kibdelosporangium.

11. The process according to Claim 10 wherein the actinomycete is selected from:

(a) Streptomyces albogriscolus,
(b) Streptomyces paucisporogenes,
(c) Streptomyces hygroscopius,
(d) Streptomyces viridochromogenes,
(e) Planomonospora parontospora, and
(f) Kibdelosporangium aridum.

12. The process according to Claim 1 wherein the mixture of the compound of Formula (4) with the contaminant of Formula (1) is produced by direct methylation of the compound of Formula (1), and wherein the compound of Formula (4) is separated by HPLC or crystallization and is recovered.

13. The process according to Claim 12 wherein direct methylation of the compound of Formula (1) or a salt thereof comprises treatment with CH_3X and $M_1{}^+NR^1R^{2-}$, wherein:

X is:

a) chloro,
b) bromo, or
c) iodo;

$M_1{}^+$is:

a) Li^+,
b) Na^+, or
c) K^+; and

R^1 and R^2 are

a) independently C_{1-3}alkyl, or
b) R^1 and R^2 joined together form a 5 or 6 membered heterocycle such as pyrrolidine or piperidine with the nitrogen to which they are attached.

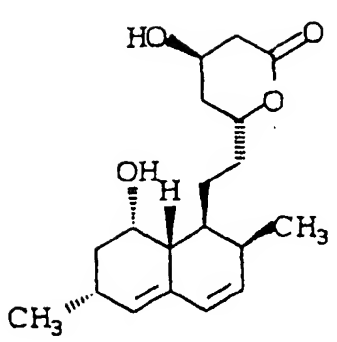
14. The process according to Claim 13 wherein X is iodo, and R^1R^2 are joined together and form pyrrolidine with the nitrogen to which they are attached and M^3 and M^4 are NH_4 or K.

15. The process according to Claim 12 wherein the product of Formula (4) is isolated and separated by crystallization.

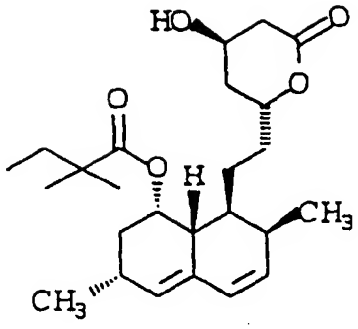
16. The process according to Claim 12 wherein the products are separated by HPLC.

17. The process of Claims 1 wherein the separation and isolation of the compounds of Formulae (2) and (4) comprise:

(a) treatment with isopropyl acetate and methanesulfonic acid to form the lactones of Formulae (3) and (5), and
(b) separation and purification of the lactones of Formulae (3) and (5) by HPLC or crystallization, and
(c) recovery of the products in the closed-ring lactone form of Formulae (3) and (5).

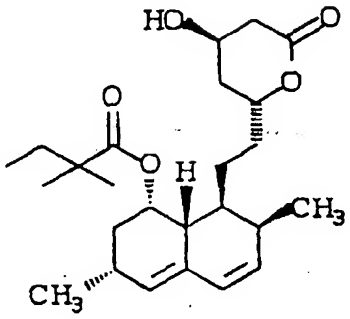


(3) Diol Lactone



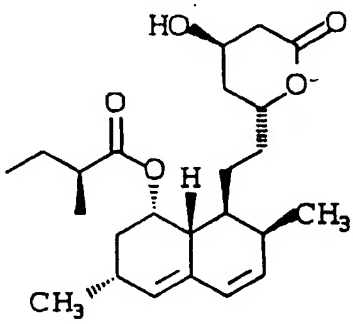
(5) Simvastatin

18. A process for preparing a compound of Formula (5)



(5) Simvastatin

or a salt thereof in recoverable amounts thereof comprising direct methylation of a compound of Formula (6)



(6) Lovastatin

by conversion of the lactone of Formula (6) to the open ring acid followed by treatment with CH_3X and $M_1{}^+NR^1R^{2-}$ wherein:

X is:

(a) chloro,
(b) bromo, or
(c) iodo;

$M_1^+$is:

(a) Li^+,
(b) Na^+, or
(c).K^+; and

R^1 and R^2 are:

(a) independently C_{1-3}alkyl, or
(b) R^1 and R^2 joined together form a 5 or 6 membered heterocycle such as pyrrolidine or piperidine with the nitrogen to which they are attached;

followed by treatment with a microbial culture capable of selectively cleaving the lovastatin 2-methylbutyryloxy side chain or a hydrolase derived from the microbial culture, followed by lactonization, and separation by HPLC or crystallization and recovery of the product of Formula (5).

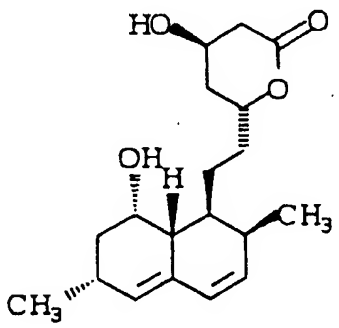
19. The process according to Claim 18 wherein X is iodo, and R^1R^2 are joined together and form pyrrolidine with the nitrogen to which they are attached and M^3 and M^4 are NH_4 or K.

20. The process according to Claim 18 wherein the product of Formula (4) is isolated and separated by crystallization.

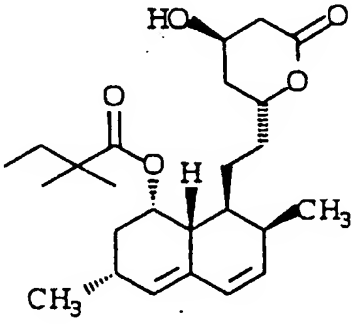
21. The process according to Claim 19 wherein the products are separated by HPLC.

22. The process of Claim 21 wherein the mixture is treated with a purified form of the hydrolase of Clonostachys compactiuscula (ATCC 74178 or 38009).

23. The process according to Claim 21 wherein lactonization comprises treatment with isopropyl acetate and methanesulfonic acid to form the lactones of Formulae (3) and (5).



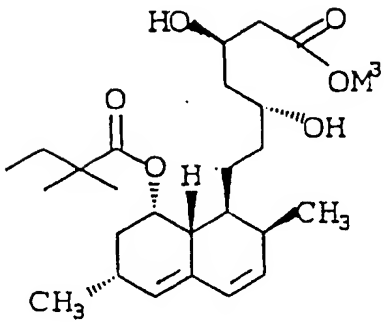
(3) Diol Lactone



(5) Simvastatin

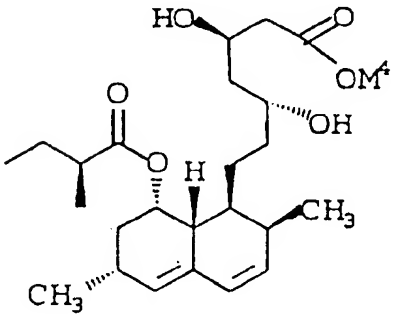
Patentansprüche

1. Verfahren zur Abtrennung einer Verbindung der Formel (4)



(4) Simvastatinsäure/salz

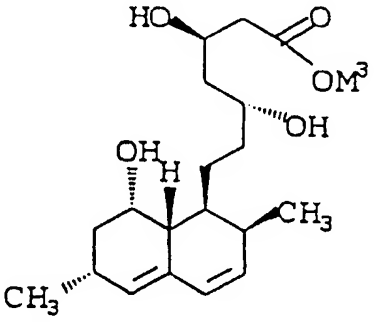
aus einer Mischung davon mit einer Verunreinigung der Formel (1)



(1) Lovastatinsäure/salz

welches umfaßt:

Behandlung der Mischung der Verbindungen mit einer mikrobiellen Kultur, welche zur selektiven Spaltung der 2-Methylbutyryloxy-Seitenkette von Lovastatin imstande ist, oder mit einer Hydrolase, die von der mikrobiellen Kultur stammt, um die Verbindung der Formel (1) in die Verbindung der Formel (2) zu überführen,



(2) Triolsäure/salz

und Abtrennung und Isolierung der Verbindungen der Formel (4) und Formel (2) in Form der offenen Säure, des

Salzes oder des Laktons, wobei
M^3 und M^4 unabhängig

(a) H,
(b) Li, Na oder K,
(c) Ca oder Mg,
(d) Al, Fe, Zn, Cu, Ni oder Co,
(e) Arginin, Lysin, α,β-Diaminobuttersäure oder Ornithin,
(f) t-Octylamin, Dibenzylamin, Ethylendiamin, Morpholin oder Tris(hydroxymethyl)aminomethan oder
(g) NH_4 darstellen.

2. Verfahren nach Anspruch 1, worin die mikrobielle Kultur eine Pilzkultur ist.

3. Verfahren nach Anspruch 2, worin der Pilz ausgewählt ist aus den Gattungen

(a) Clonostachys,
(b) Emericella,
(c) Diheterospora,
(d) Humicola,
(e) Dichotomonyces,
(f) Neocosmospora,
(g) Scopulariopsis,
(h) Xylogone,
(i) Torulomyces und
(j) Thievela.

4. Verfahren nach Anspruch 3, worin der Pilz

(a) Clonostachys compactiuscula,
(b) Monascus ruber,
(c) Mortierella isabellina,
(d) Emericella unguis,
(e) Diheterospora chlamydosporia,
(f) Humicola fuscoatra,
(g) Dichotomomyces cejpii,
(h) Neocosmospora africana,
(i) Xylogone sphaerospora,
(j) Torulomyces ragena,
(k) Thielavia fimeti,
(l) Aspergillus unguis,
(m) Mucor circinelloides,
(n) Fusarium solani,
(o) Penicillium chrysogenum,
(p) Aspergillus clavatus,
(q) scopulariopsis communis,
(r) Gilmaniella humicola,
(s) Mucor bainieri,
(t) Tricharus spiralis oder
(u) Chaetomium cochliodes ist.

5. Verfahren nach Anspruch 4, worin der Pilz

(a) Clonostachys compactiuscula,
(b) Humicola fuscoatra,
(c) Neocosmospora africana,
(d) Xylogone sphaerospora oder
(e) Scopulariopsis communis ist.

6. Verfahren nach Anspruch 5, worin der Pilz Clonostachys compactiuscula (ATCC 74178 oder 38009) oder eine Mutante davon ist.

7. Verfahren nach Anspruch 4, worin die Mischung mit einer gereinigten Form der von der betreffenden Kultur stammenden Hydrolase behandelt wird.

8. Verfahren nach Anspruch 1, worin die mikrobielle Kultur eine Bakterienkultur ist.

9. Verfahren nach Anspruch 8, worin das Bakterium ein Aktinomyzet ist.

10. Verfahren nach Anspruch 9, worin der Aktinomyzet ausgewählt ist aus den Gattungen

(a) Streptomyces,
(b) Planomonospora und
(c) Kibdelosporangium.

11. Verfahren nach Anspruch 10, worin der Aktinomyzet ausgewählt ist aus

(a) Streptomyces albogriscolus,
(b) Streptomyces paucisporogenes,
(c) Streptomyces hygroscopius,
(d) Streptomyces viridochromogenes,
(e) Planomonospora parontospora und
(f) Kibdelosporangium aridum.

12. Verfahren nach Anspruch 1, worin die Mischung der Verbindung der Formel (4) mit der Verunreinigung der Formel (1) durch direkte Methylierung der Verbindung der Formel (1) hergestellt wird und worin die Verbindung der Formel (4) durch HPLC oder Kristallisation abgetrennt und gewonnen wird.

13. Verfahren nach Anspruch 12, worin die direkte Methylierung der Verbindung der Formel (1) oder eines Salzes davon die Behandlung mit CH_3X und $M_1{}^+NR^1R^{2-}$ umfaßt, wobei

X a) Chlor, b) Brom oder c) Iod ist;
$M_1{}^+$ a) Li^+, b) Na^+ oder c) K^+ ist; und
R^1 und R^2 a) unabhängig C_{1-3}-Alkyl sind oder
b) R^1 und R^2 miteinander verbunden mit dem Stickstoffatom, an das sie gebunden sind, einen 5- oder 6-gliedrigen Heterocyclus wie Pyrrolidin oder Piperidin bilden.

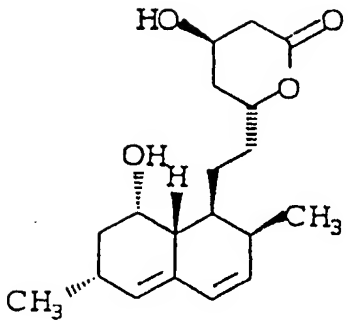
14. Verfahren nach Anspruch 13, worin X Iod ist und R^1 R^2 miteinander verbunden sind und mit dem Stickstoffatom, an das sie gebunden sind, Pyrrolidin bilden und M^3 und M^4 NH_4 oder K sind.

15. Verfahren nach Anspruch 12, worin das Produkt der Formel (4) isoliert und durch Kristallisation abgetrennt wird.

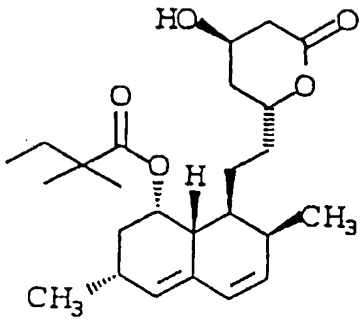
16. Verfahren nach Anspruch 12, worin die Produkte durch HPLC abgetrennt werden.

17. Verfahren nach Anspruch 1, worin die Abtrennung und Isolierung der Verbindungen der Formeln (2) und (4) umfaßt:

(a) Behandlung mit Isopropylacetat und Methansulfonsäure zur Bildung der Laktone der Formeln (3) und (5), und
(b) Abtrennung und Reinigung der Laktone der Formeln (3) und (5) durch HPLC oder Kristallisation, und
(c) Gewinnung der Produkte in der ringgeschlossenen Laktonform der Formeln (3) und (5).

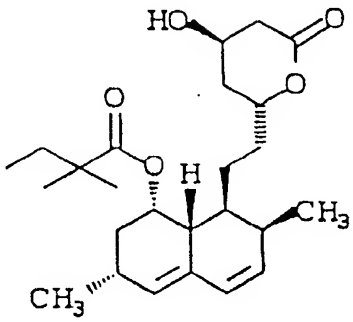


(3) Diollakton



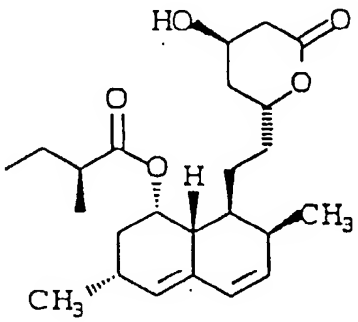
(5) Simvastatin

18. Verfahren zur Herstellung einer Verbindung der Formel (5)



(5) Simvastatin

oder eines Salzes davon in isolierbaren Mengen, welches umfaßt die direkte Methylierung einer Verbindung der Formel (6)



(6) Lovastatin

durch Überführung des Laktons der Formel (6) in die ringoffene Säure, gefolgt von einer Behandlung mit CH_3X und $M_1{}^+NR^1R^{2-}$, wobei

X a) Chlor, b) Brom oder c) Iod ist;
$M_1{}^+$ a) Li^+, b) Na^+ oder c) K^+ ist; und

R^1 und R^2 a) unabhängig C_{1-3}-Alkyl sind oder
b) R^1 und R^2 miteinander verbunden mit dem Stickstoffatom, an das sie gebunden sind, einen 5- oder 6-gliedrigen Heterocyclus wie Pyrrolidin oder Piperidin bilden;

gefolgt von einer Behandlung mit einer mikrobiellen Kultur, die zur selektiven Spaltung der 2-Methylbutyryloxy-Seitenkette von Lovastatin imstande ist, oder einer Hydrolase, die von der mikrobiellen Kultur stammt, gefolgt von Laktonisierung und Abtrennung durch HPLC oder Kristallisation und Gewinnung des Produkts der Formel (5).

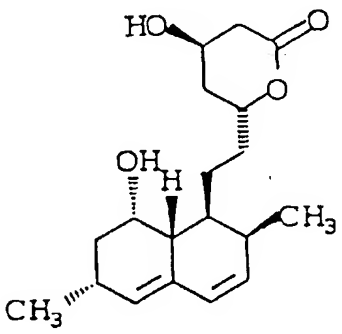
19. Verfahren nach Anspruch 18, worin X Iod ist und R^1 R^2 miteinander verbunden sind und mit dem Stickstoffatom, an das sie gebunden sind, Pyrrolidin bilden und M^3 und M^4 NH_4 oder K sind.

20. Verfahren nach Anspruch 18, worin das Produkt der Formel (4) isoliert und durch Kristallisation abgetrennt wird.

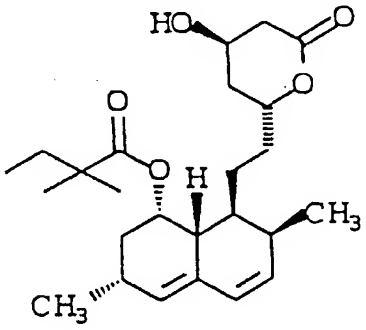
21. Verfahren nach Anspruch 19, worin die Produkte durch HPLC abgetrennt werden.

22. Verfahren nach Anspruch 21, worin die Mischung mit einer gereinigten Form der Hydrolase von Clonostachys compactiuscula (ATCC 74178 oder 38009) behandelt wird.

23. Verfahren nach Anspruch 21, worin die Laktonisierung die Behandlung mit Isopropylacetat und Methansulfonsäure zur Bildung der Laktone der Formeln (3) und (5) umfaßt.



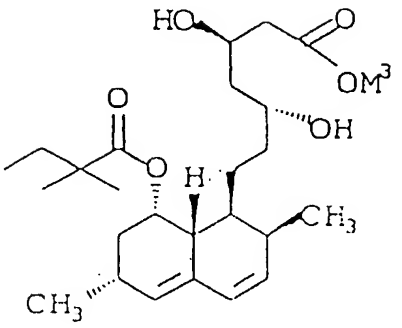
(3) Diollakton



(5) Simvastatin

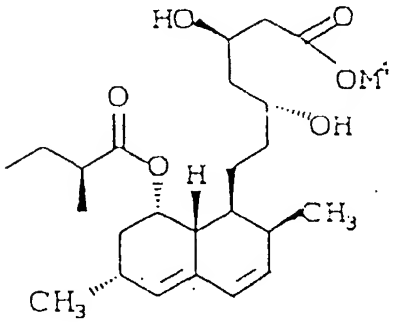
Revendications

1. Procédé de séparation d'un composé de formule (4)



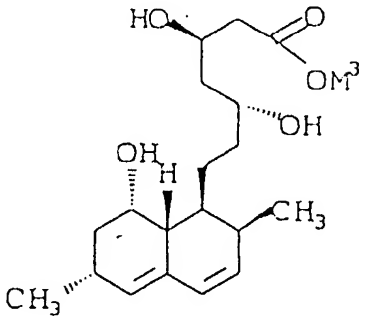
(4) Simvastatine acide/sel

à partir d'un de ses mélanges avec un produit de contamination de formule (1)



(1) Lovastatine acide/sel

qui comprend:
le traitement du mélange des composés avec une culture microbienne capable de scinder sélectivement la chaîne latérale 2-méthylbutyryloxy de la lovastatine, ou avec une hydrolase dérivée de la culture microbienne pour transformer le composé de formule (1) en composé de formule (2).



(2) Triol acide/sel

et la séparation et l'isolement des composés de formule (4) et de formule (2) sous la forme lactone, sel ou acide ouvert, où : M^3 et M^4 représentent indépendamment :

(a) H,
(b) Li. Na ou K,
(c) Ca ou Mg,
(d) Al. Fe, Zn, Cu, Ni ou Co,
(e) l'arginine, la lysine, l'acide α,β-diaminobutyrique ou l'ornithine,
(f) la t-octylamine, la dibenzylamine, l'éthylène-diamine, la morpholine ou le tris(hydroxyméthyl)-aminométhane, ou
(g) NH_4.

2. Procédé selon la revendication 1, dans lequel la culture microbienne est une culture fongique.

3. Procédé selon la revendication 2, dans lequel le champignon est choisi parmi les genres

(a) Chlonostachys,
(b) Emericella,
(c) Diheterospora,
(d) Humicola,
(e) Dichotomomyces,
(f) Neocosmospora,
(g) Scopulariopsis
(h) Xylogone,
(i) Torulomyces, et
(j) Thievela.

4. Procédé selon la revendication 3, dans lequel le champignon est :

(a) Clonostachys compactiuscula,
(b) Monascus ruber,
(c) Mortierella isabellina,
(d) Emericella unguis,
(e) Diheterospora chlamydosporia,
(f) Humicola fuscoatra,
(g) Dichlotomomyces cejpii,
(h) Neocosmospora africana,
(i) Xylogone sphaerospora,
(j) Torulomyces ragena
(k) Thielavia fimeti,
(l) Aspergillus unguis,
(m) Mucor circinelloides,
(n) Fusarium solani,
(o) Penicillium chrysogenum,
(p) Aspergillus clavatus,
(q) Scopulariopsis communis,
(r) Gilmaniella humicola,
(s) Mucor bainieri,
(t) Tricharus spiralis, ou
(u) Chaetomium cochliodes.

5. Procédé selon la revendication 4, dans lequel le champignon est :

(a) Clonostachys compactiuscula,
(b) Humicola fuscoatra,
(c) Neocosmospora africana
(d) Xylogone sphaerospora,
ou (e) Scopulariopsis communis.

6. Procédé selon la revendication 5, dans lequel le champignon est Clonostachys compactiuscula (ATCC 74178 ou 38009) ou un de ses mutants.

7. Procédé selon la revendication 4, dans lequel on traite le mélange avec une forme purifiée de l'hydrolase qui provient de la culture.

8. Procédé selon la revendication 1, dans lequel la culture microbienne est une culture bactérienne.

9. Procédé selon la revendication 8, dans lequel la bactérie est un actinomycète.

10. Procédé selon la revendication 9, dans lequel l'actinomycète est choisi parmi les genres :

(a) Streptomyces,
(b) Planomonospora, et
(c) Kibdelosporangium.

11. Procédé selon la revendication 10, dans lequel l'actinomycète est choisi parmi :

(a) Streptomyces albogriscolus,
(b) Streptomyces paucisporogènes,
(c) Streptomyces hygroscopius
(d) Streptomyces viridochromogenes,
(e) Planomonospora parontospora, et
(f) Kibdelosporangium aridum.

12. Procédé selon la revendication 1, dans lequel le mélange du composé de formule (4) avec le produit de contamination de formule (1) est produit par méthylation directe du composé de formule (1), et dans lequel le composé de formule (4) est séparé par CLHP ou cristallisation et récupéré.

13. Procédé selon la revendication 12, dans lequel la méthylation directe du composé de formule (1) ou d'un de ses sels comprend le traitement avec CH_3X et $M_1{}^+NR^1R^{2-}$, où :

X représente :

a) un atome de chlore,
b) un atome de brome, ou
c) un atome d'iode ;

$M_1{}^+$ représente :

a) Li^+,
b) Na^+, ou
c) K^+ ; et

R^1 et R^2 représentent

a) indépendamment un groupe alkyle en C_{1-3}, ou
b) R^1 et R^2 reliés conjointement avec l'azote auquel ils sont fixés, forment un hétérocycle à 5 ou 6 chainons tel que la pyrrolidine ou la pipéridine.

14. Procédé selon la revendication 13, dans lequel X représente un atome d'iode, et R^1 R^2 sont reliés ensemble et forment un noyau pyrrolidine avec l'atome d'azote auquel ils sont fixés et M^3 et M^4 représentent NH_4, ou K.

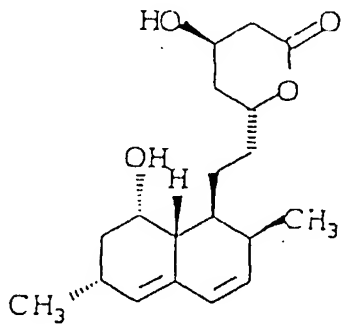
15. Procédé selon la revendication 12, dans lequel le produit de formule (4) est isolé et séparé par cristallisation.

16. Procédé selon la revendication 12, dans lequel les produits sont séparés par CLHP.

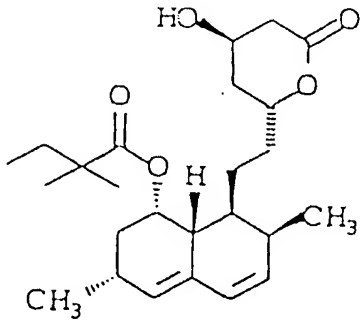
17. Procédé selon la revendication 1, dans lequel la séparation et l'isolement des composés de formules (2) et (4)

comprennent :

a) le traitement avec l'acétate d'isopropyle et l'acide méthanesulfonique pour former les lactones de formules (3) et (5), et
b) la séparation et la purification des lactones de formules (3) et (5) par CLHP ou cristallisation, et
c) la récuparation des produits sous la forme lactone à cycle fermé de formules (3) et (5)

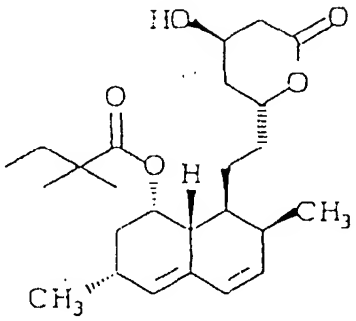


(3) Diol lactone



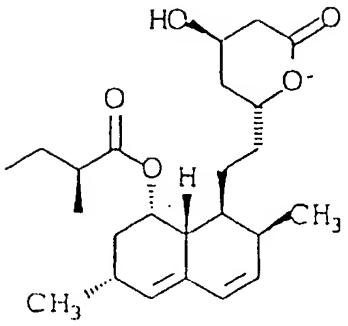
(5) Simvastatine

18. Procédé de préparation d'un composé de formule (5)



(5) Simvastatine

ou d'un de ses sels, en des quantités récupérables de celui-ci, qui comprend la méthylation directe d'un composé de formule (6)



(6) Lovastatine

par conversion de la lactone de formule (6) en l'acide à cycle ouvert suivie du traitement avec CH_3X et $M_1{}^+NR^1R^2{}^-$ où

X représente

(a) un atome de chlore,
(b) un atome de brome, ou
(c) un atome d'iode,

$M_1{}^+$ représente

(a) Li^+,
(b) Na^+, ou
(c) K^+ ; et

R^1 et R^2 représentent :

(a) indépendamment un groupe alkyle en C_{1-3}, ou
(b) R^1 et R^2 reliés ensemble forment avec l'atome d'azote auquel ils sont fixés, un hétérocycle à 5 ou 6 chaînons tel que pyrrolidine ou pipéridine ;

suivie du traitement avec une culture microbienne capable de scinder sélectivement la chaîne latérale 2-méthylbutyryloxy de la lovastatine ou une hydrolase dérivée de la culture microbienne, suivi de la lactonisation et de la séparation par CLHP ou cristallisation et de la récupération du produit de formule (5).

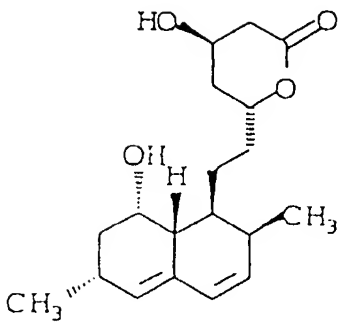
19. Procédé selon la revendication 18, dans lequel X représente un atome d'iode et R^1 et R^2 sont liés ensemble et forment un noyau pyrrolidine avec l'atome d'azote auquel ils sont fixés et M^3 et M^4 représentent NH_4 ou K.

20. Procédé selon la revendication 18, dans lequel le produit de formule (4) est isolé et séparé par cristallisation.

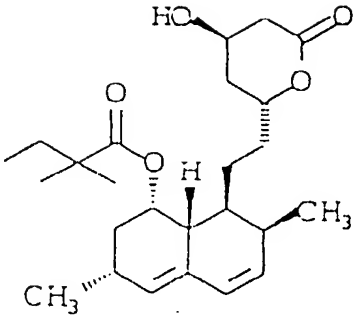
21. Procédé selon la revendication 19, dans lequel les produits sont séparés par CLHP.

22. Procédé selon la revendication 21, dans lequel le mélange est traité avec une forme purifiée de l'hydrolase de Clonostachys compactiuscula (ATCC 74178 ou 38009).

23. Procédé selon la revendication 21, dans lequel la lactonisation comprend le traitement avec l'acétate d'isopropyle et l'acide méthanesulfonique pour former les lactones de formules (3) et (5),



(3) Diol lactone



(5) Simvastatine